

Full Length Research Paper

# Micropropagation of superior eucalyptus hybrids FRI-5 (*Eucalyptus camaldulensis* Dehn x *E. tereticornis* Sm) and FRI-14 (*Eucalyptus torelliana* F.V. Muell x *E. citriodora* Hook): A commercial multiplication and field evaluation

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Micropropagation protocols for multiplication of highly important *Eucalyptus* hybrids have been achieved. Two *Eucalyptus* hybrids namely as FRI-5 (*Eucalyptus camaldulensis* Dehn x *Eucalyptus tereticornis* Sm) and FRI-14 (*Eucalyptus torelliana* F. V. Muell x *Eucalyptus citriodora* Hook) selected for micropropagation using nodal segments as explants from the mature trees (30 - 32 years old) for the two hybrids. These *Eucalyptus* hybrids were produced by Forest Research Institute, Dehradun, in the early 1960 - 70 through controlled crossing. These are available in limited number and need multiplication true to type for field - testing to ascertain its superiority under varied agro - climatic conditions. These hybrids showed 3 - 5 superior in terms of total biomass and wood volume when compared to their parents. Clonal cultures were established and multiplied on MS medium supplemented different concentration of BAP either alone or in with combination of auxins i.e. IBA/NAA. MS medium was found to be effective and suitable for all the experiments during plant production. 85 - 92% rooting was achieved on half strength MS medium supplemented with IBA. 90 - 98% of the plantlets survived hardening and acclimatization prior to field transfer. Parameters like height, DBH, clear bole length and self pruning capability of both the hybrids were recorded up to three years to identify the suitability of these hybrids in different varied climatic zones of Uttarakhand.

**Key words:** *Eucalyptus* hybrids FRI-5 and FRI-14, field testing, diameter at breast height, micropropagation.

## INTRODUCTION

*Eucalypts* are credited with high growth rate and multiple uses and are planted extensively. *Eucalyptus* produces some of the heaviest, hardest and most durable wood, thus making this genus the most valuable source of hardwood in the world. To meet the increasing demand

for timber in the future, fast growing *Eucalyptus* plantations are preferred. *Eucalyptus* hybrids have been a widely planted species in India owing to its adaptability in different eco-climatic zones. Hybridization is a known process by which the desirable traits of the two parents may be combined in F<sub>1</sub> offspring. It also offers a means to capture the benefits of hybrid vigour (heterosis), which is often manifested in certain specific parental species combinations in hybrids. Development of hybrids, their clonal multiplication and post juvenile character studies still remains a serious problem due to various reasons. Forest research Institute, Dehradun, has 13 mature F<sub>1</sub> *Eucalyptus* hybrid combinations. At Forest Research Institute, Dehradun, work on hybridization in *Eucalyptus*

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**Abbreviations:** BAP, 6-Benzylaminopurine; NAA,  $\alpha$ -naphthalene acetic acid; IBA, indole-3- butyric acid; MS, Murashige and Skoog's (1962) medium; DBH, diameter at breast height.

**Table 1.** Effect of cytokinin (BAP) in MS medium on axillary bud induction using nodal segments of FRI-5 (data recorded after 5 weeks).

BAP (mg/l)	Response (%)	Mean shoot number	Mean shoot length (cm)
Control	12.50± 0.29	0.50 ± 0.22	0.13 ± 0.06
0.1	20.83 ± 0.48	0.83 ± 0.31	0.23 ± 0.09
0.5	45.83± 0.48	1.83 ± 0.31	0.77 ± 0.04
1.0	83.33 ± 0.19	3.33 ± 0.33	0.89 ± 0.03
1.5	66.66 ± 0.38	2.67 ± 0.33	0.92±0.04
2.0	41.66± 0.38	1.67 ± 0.21	0.55 ± 0.03
2.5	33.33 ± 0.19	1.33 ± 0.21	0.38 ± 0.02
Significance	***	***	***
CD at 5%	1.09	0.81	0.14

was initiated during 1970s (Venkatesh and Sharma, 1977c). Based on cross - ability pattern, studies were initiated to produce controlled hybrids as well as natural hybrids from half-sib progenies raised from seeds collected from stands of two intercrossable species growing in vicinity to each other. The hybrids are artificially produced or spontaneously selected from New Forest area at Forest Research Institute campus, Dehradun. Out of these different interspecific hybrids developed, two hybrids were selected for study i.e. FRI-5 and FRI-14. Both the *Eucalyptus* hybrids FRI-5 and FRI-14 were selected for present study has shown superior character over their parental combination. They have also displayed a very high degree of hybrid vigour and proved 3-5 times superior in growth parameters than the parent combinations. However, when F<sub>2</sub> population raised through seeds a lot of segregation was observed which had reduced the average yield per unit area per unit time. These hybrids produced higher biomass than the parental progenies and the Mysore Gum (Venkatesh and Sharma, 1977a,b). We developed micropropagation protocol for cloning and large scale production as non-conventional methods of clonal propagation for commercial cultivation of these valuable hybrids of *Eucalyptus*.

## MATERIALS AND METHODS

Nodal shoots segments containing axillary buds were harvested from the two natural F<sub>1</sub> hybrid of *Eucalyptus*, that is FRI-5 (*E. camaldulensis* Dehn x *E. tereticornis* Sm) and FRI-14 (*E. torelliana* F. V. Muell x *E. citriodora* Hook). The explants were harvested from newly developed fresh shoots during early in the morning and proved to be the best time for explant collection. It was found that the explants collected during January to February and August to September were the best for *in vitro* studies as they showed least phenolic exudation and gave 65 - 70% bud break response as compared to other months.

## RESULTS OF *IN VITRO* STUDIES

### Collection and Preparation of Explant

The nodal shoot segments containing axillary buds were

collected from 30-32 years old mature trees of *Eucalyptus* hybrids FRI-5 and FRI-14 growing at New Forest experimental field of Forest Research Institute, Dehradun. Axillary bud/nodal segments measuring 2-3 cm were cut. Explant treated with bavistin (1%) and antibiotics (Streptomycin and Chloramphenicol) for 3-5 minutes followed by surface sterilization with (0.15%) HgCl<sub>2</sub> (12 min for FRI-5 and 10 min for FRI-14) was found to be very effective in controlling 78 - 80% contamination with good survival rate of 65 - 75%.

### Axillary bud/Shoot Initiation

Four nutrient media viz. MS medium (Murashige and Skoog, 1962), Woody Plant Medium (Llyod and McCown, 1980), B5 medium (Gamborg et al., 1968) and SH medium (Schenk and Hildebrandt, 1972) were tested for the establishment of aseptic cultures from axillary buds and for shoot multiplication. All the media containing 2% sucrose solidified (in case of FRI-5) and 3% (in case of FRI-14) with 0.6% bacteriological agar and supplemented with different concentrations of BAP individually and along with NAA was used for the establishment of bud induction. Initiation of buds started without an intervening callus phase within 3 weeks from the date of inoculation of the nodal segments. Among the four media, MS medium was found to be very effective for axillary bud induction and shoot initiation for both the hybrids. Nodal segments of FRI-5 when cultured on MS medium supplemented with 1.0 mg/l BAP, gave 83.33% bud break. In FRI-14, 70.83% bud break response was obtained on (MS+1.5 mg/l BAP + 0.5 mg/l NAA (Tables 1 and 2).

### Shoot multiplication

Axillary buds were inoculated onto the MS media supplemented with different concentrations of BAP alone and in combination with NAA. Out of the different media combinations tested, best shoot multiplication occurred on MS + 1.0 mg/l BAP + 0.1 mg/l IBA in FRI-5 and on MS + 1.0

**Table 2.** Effect of hormonal interaction (BAP+NAA) in MS medium on axillary bud induction using nodal segments of FRI-14 (data recorded after 5 weeks).

BAP + NAA (mg/l)	Response (%)	Mean shoot number	Mean shoot length (cm)
Control	4.16 ± 0.09	0.17 ± 0.17	0.07 ± 0.07
0.1 + 0.1	8.33 ± 0.19	0.33 ± 0.21	0.13 ± 0.08
0.1 + 0.5	12.50 ± 0.29	0.67 ± 0.21	0.40 ± 0.13
0.1 + 1.0	8.33 ± 0.19	0.67 ± 0.21	0.50 ± 0.16
0.1 + 1.5	16.67 ± 0.39	1.00 ± 0.00	0.72 ± 0.03
0.5 + 0.1	12.50 ± 0.29	1.67 ± 0.21	0.77 ± 0.01
0.5 + 0.5	20.83 ± 0.48	2.17 ± 0.17	0.82 ± 0.02
0.5 + 1.0	25.00 ± 0.58	2.83 ± 0.31	0.84 ± 0.00
0.5 + 1.5	20.83 ± 0.48	3.33 ± 0.21	0.81 ± 0.02
1.0 + 0.1	29.16 ± 0.09	3.83 ± 0.31	1.01 ± 0.04
1.0 + 0.5	41.66 ± 0.38	4.83 ± 0.31	1.50 ± 0.04
1.0 + 1.0	37.50 ± 0.29	4.00 ± 0.37	0.94 ± 0.00
1.0 + 1.5	29.16 ± 0.09	3.33 ± 0.21	0.99 ± 0.02
1.5 + 0.1	45.83 ± 0.48	3.00 ± 0.26	1.05 ± 0.02
1.5 + 0.5	70.83 ± 0.48	5.67 ± 0.33	1.86 ± 0.01
1.5 + 1.0	58.33 ± 0.19	4.50 ± 0.22	1.68 ± 0.07
1.5 + 1.5	54.16 ± 0.09	3.67 ± 0.21	1.58 ± 0.01
2.0 + 0.1	50.00 ± 0.58	2.67 ± 0.33	1.26 ± 0.01
2.0 + 0.5	41.34 ± 0.19	2.00 ± 0.26	1.16 ± 0.03
2.0 + 1.0	37.50 ± 0.29	1.67 ± 0.21	0.92 ± 0.02
2.0 + 1.5	33.33 ± 0.19	1.50 ± 0.22	0.73 ± 0.02
2.5 + 0.1	29.16 ± 0.09	1.33 ± 0.21	0.50 ± 0.03
2.5 + 0.5	20.83 ± 0.48	1.33 ± 0.21	0.35 ± 0.03
2.5 + 1.0	12.50 ± 0.29	0.83 ± 0.17	0.17 ± 0.03
2.5 + 1.5	8.33 ± 0.19	0.67 ± 0.21	0.13 ± 0.04
Significance	***	***	***
CD at 5%	0.95	0.67	0.15

\*\*\*Significance at 0.1%, ± values represent the standard error.

**Table 3.** Effect of hormonal interaction cytokinin and auxin (BAP + IBA) in MS medium on *in vitro* shoot multiplication of FRI-5 (data recorded after 5 weeks).

BAP + IBA (mg/l)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
Control	10.33 ± 0.42	0.50 ± 0.03	2.07 ± 0.08
1.0 + 0.1	57.17 ± 1.38	1.51 ± 0.04	11.43 ± 0.28
1.0 + 0.5	45.83 ± 0.75	1.33 ± 0.08	9.17 ± 0.15
***	37.17 ± 0.83	0.61 ± 0.06	7.43 ± 0.17
1.0 + 1.0	24.33 ± 1.20	0.44 ± 0.01	4.87 ± 0.24
1.0 + 1.5	23.80 ± 0.96	1.12 ± 0.07	4.70 ± 0.19
Significance	***	***	***
CD at 5%	2.81	0.16	0.56

mg/l BAP in case of FRI-14. Multiple shoots were obtained after 5 weeks in both the hybrids (Tables 3 and 4). A regular sub-culturing was carried out every 5 weeks on fresh medium. Cultures were incubated at 25 ± 2°C 16

hours in light (illuminated by 40 watt fluorescent tubes, 1200 lux) and for 8 hours in dark cycle irradiance by cool fluorescent tubes. The cultures were regularly transferred into fresh medium to check the browning of cultures.

**Table 4.** Effect of cytokinin (BAP) in MS medium on *in vitro* shoot multiplication of FRI-14 (data recorded after 5 weeks).

BAP(mg/l)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
Control	22.33 ± 1.12	1.07 ± 0.02	5.58 ± 0.28
0.5	33.50 ± 1.26	2.01 ± 0.12	8.38 ± 0.31
1.0	51.33 ± 2.35	3.03 ± 0.16	11.83 ± 0.16
1.5	40.80 ± 1.40	2.63 ± 0.05	6.80 ± 0.15
2.0	30.17 ± 0.91	1.97 ± 0.06	5.20 ± 0.12
2.5	20.50 ± 0.62	1.46 ± 0.05	3.53 ± 0.18
Significance	***	***	***
CD at 5%	4.00	0.26	0.42

\*\*\*Significance at 0.1%; ± values represent the standard error.

**Table 5.** Effect of strength of different basal media supplemented with 1.0 mg/l BAP + 0.1mg/l IBA in MS medium on *in vitro* shoot multiplication of FRI-5 (data recorded after 5 weeks).

Media	Mean shoot number	Mean shoot length (cm)	Multiplication rate
MS 1 x	52.17 ± 0.60	1.14 ± 0.04	10.43 ± 0.12
B <sub>5</sub> 1 x	11.83 ± 0.79	1.10 ± 0.01	2.37 ± 0.16
WPM 1 x	29.17 ± 0.95	0.75 ± 0.05	5.83 ± 0.19
SH 1 x	11.50 ± 0.76	0.60 ± 0.04	2.30 ± 0.15
Significance	***	***	***
CD at 5 %	2.06	0.10	0.41

**Table 6.** Effect of strength of basal medium supplemented with 1.0 mg/l BAP in MS medium on *in vitro* shoot multiplication of FRI-14 (data recorded after 5 weeks).

Media	Mean shoot number	Mean shoot length (cm)	Multiplication rate
MS 1 x	53.67 ± 1.05	3.14 ± 0.02	10.73 ± 0.21
B <sub>5</sub> 1 x	14.83 ± 0.79	3.56 ± 0.00	2.97 ± 0.16
WPM 1 x	30.00 ± 0.58	0.76 ± 0.00	6.00 ± 0.12
SH 1 x	11.17 ± 0.48	1.59 ± 0.03	2.23 ± 0.10
Significance	***	***	***
CD at 5 %	1.96	0.03	0.39

\*\*\*Significance at 0.1%; ± values represent the standard error.

### Effect of different basal media and their strength

Maximum shoot multiplication rate (9 - 12 folds) was obtained on MS medium supplemented with 1.0 mg/l BAP + 0.1 mg/l IBA mg/l in case of FRI-5 and in case of FRI-14 maximum shoot multiplication rate (10 - 12 folds) was obtained on MS medium supplemented with 1.0 mg/l BAP. Amongst all media, shoot multiplication was highest (found optimum) on MS medium with a shoot multiplication rate of 10 - 12 folds (Tables 5 and 6). It was found that maximum shoot multiplication rate was obtained on full strength MS medium. *In vitro* shoot elongation was

obtained on both ½ and ¼ strength MS medium without PGR.

### Regeneration of roots

After elongation individual shoots measuring 2.5 to 3.0 cm were inoculated on half strength MS medium supplemented with IBA (0.1-2.0 mg/l) alone and in the combination with NAA (0.1-2.0 mg/l) for rooting. 87.50% rooting was achieved in FRI-5 on ½ MS supplemented with IBA (1.0 mg/l) and 91.66% rooting was achieved in

**Table 7.** Effect of IBA on rooting of *in vitro* shoot on half strength MS medium of FRI-5 (data recorded after 5 weeks).

IBA (mg/l)	Rooting (%)	Mean root number	Mean root length (cm)
Control	12.5 ± 0.29	0.50 ± 0.22	0.35 ± 0.16
0.1	20.83 ± 0.48	8.67 ± 0.56	0.73 ± 0.05
0.5	66.66 ± 0.38	17.17 ± 0.48	1.02 ± 0.03
1.0	87.50 ± 0.29	20.17 ± 0.60	2.34 ± 0.04
1.5	70.83 ± 0.48	18.83 ± 0.70	1.47 ± 0.05
2.0	54.16 ± 0.09	11.33 ± 0.67	1.30 ± 0.03
Significance	***	***	***
CD at 5%	1.11	1.62	0.22

**Table 8.** Effect of IBA on rooting of *in vitro* shoot on half strength MS medium of FRI-14 (Data recorded after 5 weeks).

IBA (mg/l)	Rooting (%)	Mean root number	Mean root length (cm)
Control	12.5 ± 0.29	0.50 ± 0.22	0.35 ± 0.16
0.1	45.83 ± 0.68	8.67 ± 0.56	0.73 ± 0.05
0.5	91.66 ± 0.86	17.17 ± 0.48	1.02 ± 0.03
1.0	79.16 ± 0.24	20.17 ± 0.60	2.34 ± 0.04
1.5	62.50 ± 0.46	18.83 ± 0.70	1.47 ± 0.05
2.0	41.66 ± 0.54	11.33 ± 0.67	1.30 ± 0.03
Significance	***	***	***
CD at 5%	1.11	1.62	0.22

\*\*\*Significance at 0.1%; ± values represent the standard error.

FRI-14 on ½ MS supplemented with IBA (0.5 mg/l) without intervening callus phase (Tables 7 and 8).

### Acclimatization and hardening and field transfer

Micropropagated and rooted plantlets were hardened *in vitro* in liquid ¼ MS medium having 2% sucrose. Absorbent cotton soaked in this liquid medium was used for supporting root system of *in vitro* raised plantlets. Plantlets were maintained in this step for 2 weeks, transferred to mist chamber in polythene bags containing a mixture of soil, sand and manure (1:1:1) and covered with perforated polythene bags and then transferred to the net house. Holes were made in the polythene bags, which were withdrawn periodically, and the plantlets were finally transferred to the field. 85 - 95% success in field survival rate was observed in both the hybrids.

### Field plantation

All the experimental sites were leveled and at places where irrigation facility was available for irrigation of the plantation was done every fourth night. The trial was laid out in block plantation along with the control local hybrid. Field study of tissue culture raised plants of both *Euca-*

*lyptus* hybrids FRI-5 and FRI-14 was done on following aspects viz survival rate and growth parameters like height, diameter and clear bole length. So far 200 tissue culture raised plants of each hybrid were planted in field during monsoon season of 2005 in different eco-climatic zones of Uttarakhand (Table 9).

Tissue culture raised plants of FRI-5 and FRI-14 were planted in three meter apart rows and the plant to plant distance was also 3 m (monsoon year of 2005). Each treatment had 20 plants in a row. The total rows were 10. The control plants raised by locally available seeds were also maintained at the same site. Casualty replacement was done during the second year. Other cultural operations such as weeding, fertilizing and mulching were done up to 3 years. The height, collar diameter and clear bole length were taken twice a year (June and December). The survival count was also noted during this period.

### RESULT OF FIELD TRIALS

In the present study tissue culture raised cloned plants were planted at three sites of different climatic conditions of Uttarakhand. An average height of 7.5-9.5 m were observed with an average diameter of 6.5-8.5 cm and a survival rate of 90-92% at the sites of Dehradun, while at Pantnagar and Haldwani fields a survival rate of 86-90%

**Table 9.** Geographical distributions of different agro-climatic sites of Uttarakhand State for field plantation.

Plantation Site	Soil type	pH range	Altitude	Latitude	Longitude	Annual rainfall (mm)	Major climatic condition	Mean temperature
Forest Research Institute, Dehradun	Sandy Loam	6.4 - 7.5	661 - 665 m	30°N	78°E	2180	Humid sub-tropical	1 - 41 °C
G.B. Pant Agriculture and Technology University, Pant Nagar	Loamy Sand	6.6 - 6.9	243.84 m	29°3'N	79°30'E	1500 - 1600	Sub-tropical, water logged, high rainfall	3 - 43.2 °C
Tanda Range, Haldwani	Loamy Sand	6.5 - 7.1	680 - 685 m	29°13'N	79° 31'E	878	Sub-tropical, Tarai area	1.5 - 39 °C

**Table 10.** Field performance after three years of tissue cultured clones of *Eucalyptus* hybrids (FRI-5 and FRI-14) under different varied climatic conditions of Uttarakhand State.

Plantation sites	Avg. height (cm)			Avg. collar dia. (cm)			Avg. clear bole length			Volume (m <sup>3</sup> )		
	FRI-5	FRI-14	Control	FRI-5	FRI-14	Control	FRI-5	FRI-14	Control	FRI-5	FRI-14	Control
Dehradun	695.71	765.96	546.35	6.93	7.89	4.00	174.0	283.5	133.4	0.011	0.016	0.003
Pant Nagar	705.76	579.02	493.62	7.04	7.16	3.86	213.2	183.4	123.8	0.011	0.009	0.002
Haldwani	704.19	594.02	511.67	7.35	7.33	4.05	216.6	194.5	129.5	0.011	0.009	0.003
Significance	**	**	**	***	***	***	NS	NS	NS			
CD at 1%	129.04			0.617			-			-		

\*\*\* Significance at 0.1%; \*\* Significance at 1.0 %; \* Significance at 5.0%; NS- non significant.

Note: Average of 50 plants.

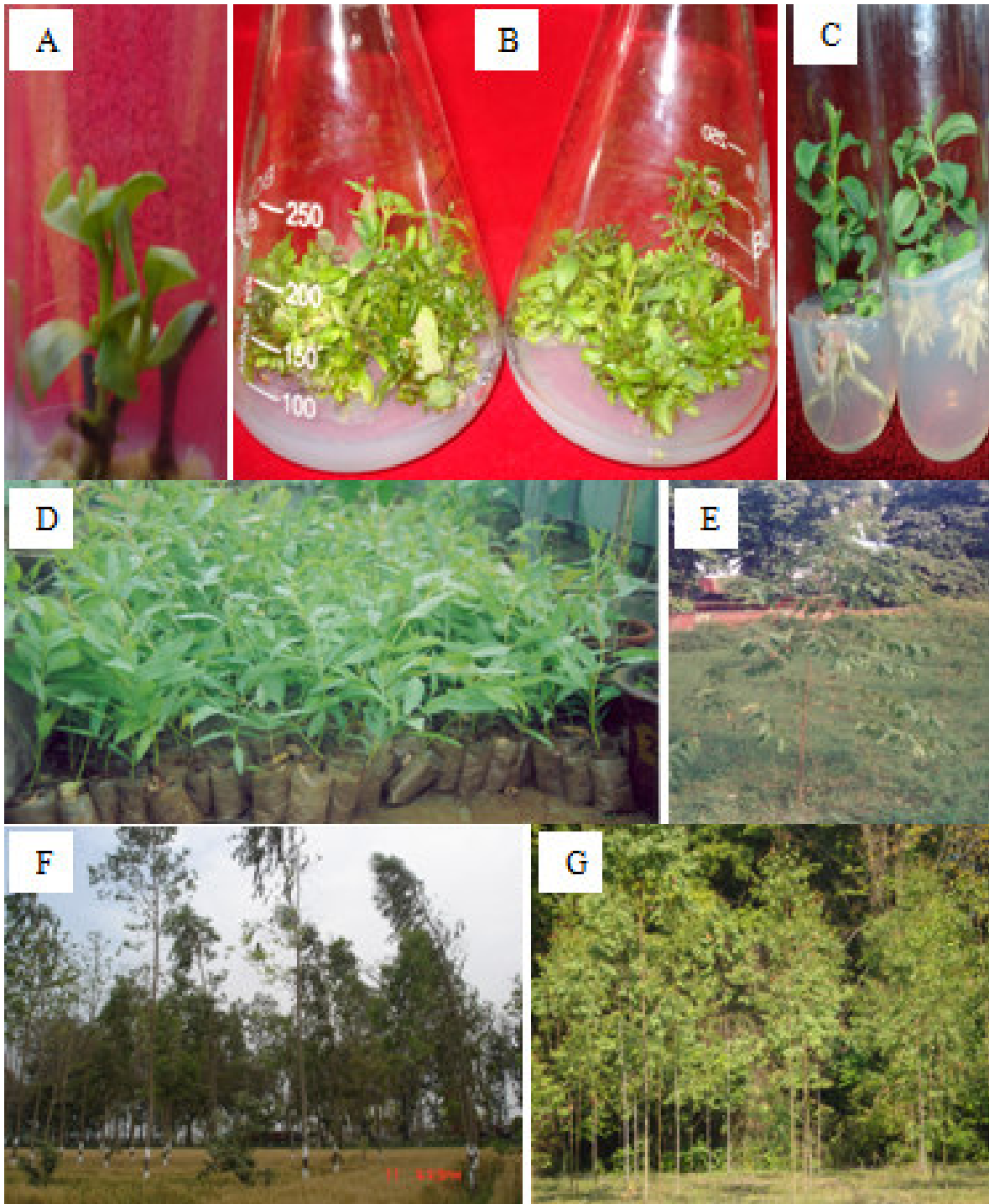
was recorded after three years of plantation. Total height and collar diameter taken on breast height (~137.0 cm) were recorded after three years and variation was observed in height and collar diameter at different plantation sites (Table 10).

The growth performance and survival percentage of both *Eucalyptus* hybrids FRI-5 and FRI-14 showed their suitability in particular environmental conditions. The analysis of survival percentage showed that the hybrids as well as the localities also when compared do not differ significantly. It indicates that localities or hybrids have no effect on survivalability of plants. Hence, it was considered to the best for commercial plantation at

these three plantation sites.

Both *Eucalyptus* hybrid FRI-5 and FRI-14 performed better for all these traits when compared with control. However, statistical analysis indicated non-significant differences among the hybrids and control for all the traits studied. The analysis of average height showed that the hybrids differ significantly but localities show no significant difference among them. The average height of the hybrids is significantly higher than the control. Like wise, the analysis of average collar diameter shows that the hybrids differ significantly but localities show no significant difference among them. The average collar diameter

of the hybrids is significantly higher than the control. While the analysis of average clear bole length shows that the hybrids as well as the localities do not differ significantly. The uniformity obtained in the plantation with micropropagated plants confirms the feasibility of using this technique for commercial scale multiplication of both *Eucalyptus* hybrids (FRI-5 and FRI-14). The overall data collected after three years shows that both FRI-5 and FRI-14 performed well and are suited to the varied climatic conditions of Dehradun, Pantnagar, Haldwani (Figures 1H-J) and also showed good self pruning up to three years of age. Now the plants are in fourth year of age.



**Figure 1.** Commercial multiplication of *Eucalyptus* hybrids FRI-5 and FRI-14 from mature tree (30 - 32 years old). (A) Axillary bud induction using nodal explant collected from mature mother plant (30 - 32 years old); (B) *In vitro* shoot multiplication in FRI-5 (after 5 weeks); (C) *In vitro* rooted plantlets; (D) Hardened and acclimatized plants in poly bag ready to field transfer; (E) 6<sup>th</sup> month old plant in field; (F and G) 3 years old field plantation at different plantation sites.

## DISCUSSION

The present investigation demonstrates the successful

multiplication by axillary meristem and field plantation at different eco-climatic zones of Uttarakhand state. Systematic field evaluation data for tissue culture raised plants

is not available. Although number of tissue culture raised plants have gone to the field but large-scale field trials is limited. Clonal plantations of *Eucalyptus* are being raised by conventional vegetative propagation method by private organizations having captive consumption (Lal, 1994). Plantation has displayed a very high degree of vigour (positive heterosis) both in height, diameter and wood quality. Data showed vigorous growth when compared with local *Eucalyptus* species in terms of total height, collar diameter and clear bole length. This may be attributed due to genetic constitution of the hybrid as well as environmental interaction constituting various physical factors like moisture content, soil pH, mean annual rainfall, mean temperature, etc. Preliminary investigation showed that hybrids vigour of the hybrids was transferred in clonal material fully and expressed even after the age of 36 months. The present trials had also shown heterotic superiority of hybrids but with variation due to variable interaction between genotype and environments. The same is reflected in these hybrids grown in the different climatic conditions as data reflected variations in the entire traits studied. Sreedhar and Rao, (1998) developed commercial scale micropropagation technique for *E. citriodora* and *E. camaldulensis* and introduced their field plantation at various locations with selected clones of *E. citriodora*. They have recorded performance after 13 months of plantation and found that micropropagated clones were distinctly superior and highly uniform. Kaur and Saxena (2002) evaluated multilocational trials of *E. tereticornis* in Haryana. They also collected parameters like height, DBH, clear bole height and self pruning capability of different clones for the identification of suitable clones for different agro climatic zones of Haryana. Biswas et al., 1999 also reported field trial of tissue cultured raised *E. tereticornis* and establishment environment factor as a key to the developmental stages in *Eucalyptus*. Johnson (1955) also reported that hybrids can express true to type vigour only under similar site of environmental conditions. Venkatesh and Sharma (1977a) reported that  $F_1$  hybrids may display varied hybrid vigour at one stage or other during their development period, thus it is necessary that the present study under different climatic condition may be carried out till maturity of these cloned hybrids. The heterotic effects of hybrids are mainly due to the specific gene combination in the hybrids. pH and moisture content from all the three sites were also recorded to see their effect on growth performance. Average pH as recorded from all the three plantation sites was in the range of 6.5-7.5 which does not affect the survival and growth performance of *Eucalyptus* (Table 9). It was found that *Eucalyptus* hybrid made satisfactory growth in soils with pH less than 9.0 and TSS less than 0.3 but failed to establish in soils of higher pH and salt content and in soils with a kankar pan. Kaushik et al. (1969) showed that *Eucalyptus* failed to grow in saline-alkali soils which have a pH above 10 and soluble salt content above 0.7% and

posses compact indurate sub soil due to kankar. Its growth was found to be arrested on the soils which have pH below 8.5 but salt content above 1.0%. Moisture content was recorded maximum as 40.21 in December and minimum as 13.34 in June. It was generally observed that under conditions of restricted soil moisture, water conserving mechanisms are in operation and the plants tend to restrict the water loss so that the growth is not seriously affected. These results showed that high rate of transpiration by *Eucalyptus* are adaptability operative under the conditions of adequate soil moisture (Rawat et al., 1984).

## Conclusion

In the present study effective and commercially viable micropropagation techniques were successfully developed for mass multiplication of two superior *Eucalyptus* hybrids FRI-5 and FRI-14. Preliminary investigation after three years of field plantation at different eco-climatic sites having varied environmental conditions showed that *Eucalyptus* hybrids FRI-5 and FRI-14 are promising in terms of height, diameter, clear bole length and self-pruning capability. However, suitability of hybrids in a particular eco-climatic zone can be determined by making continuous observations over a period of about 8-10 years. Present finding is based on a period of three years.

In the light of above findings it may be concluded that both the *Eucalyptus* hybrids FRI-5 and FRI-14 produced on large scale through tissue culture technology performed well in terms of growth parameters up to three years of age when compared to control and now can be considered as site specific plants species for all the three respective plantation sites in Uttarakhand state viz. Dehradun, Pantnagar and Haldwani.

## Summary

*Eucalyptus* hybrids are an important forest tree species under the social and commercial forestry programme with a view to get more yield per unit area. The wide spread application of *in vitro* propagation on its cost-competitiveness and is profitable only when there is an associated advantages over conventional propagation methods. The field testing of these hybrids revealed their suitability in a particular climatic zone. Also these results had shown their performance with respect to the total biomass production. The hybrid if found suitable even after 7-8 years (rotation cycle) with improved timber and oil production will be of immense value for commercial growers of *Eucalyptus*. The trial of *in vitro* raised plantlets which had completed after three years will provide requisite material for testing the hybrids for various qualitative traits for pulp and paper based industries. Beside

this clonal propagation is the only answer to tap the productive potential of these hybrids which have been achieved successfully.

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